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Invited article

Origin and Evolution of "Plants" as Deduced from Genome Information

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Phylogenetic relationships between three lineages of the primary photosynthetic eukaryotes (red algae, green plants and glaucophytes) seemed to remain unresolved because previous nuclear multigene phylogenies used the incomplete red algal gene sequences. Recently, we carried out phylogenetic analyses based on a 1525-amino-acid sequence of four concatenated nuclear genes from various lineages of only mitochondria-containing eukaryotes, using complete genome sequences from the red alga *Cyanidioschyzon merolae*. This study resolved two large monophyletic groups (groups A and B) and the basal group (Amoebozoa). Group A corresponded to the Opisthokonta (Metazoa and Fungi), whereas group B included various primary and secondary plastid-containing lineages (euglenoids, heterokonts, and apicomplexans), Ciliophora, Kinetoplastida, and Heterolobosea. The red algae represented the most basal lineage within group B. Since the single event of the plastid primary endosymbiosis was strongly suggested by other data, it was considered that the primary plastid endosymbiosis likely occurred once in the common ancestor of group B, and the primary plastids were subsequently lost in the ancestor(s) of organisms which now lack primary plastids within group B. A new concept of "Plantae" was proposed for phototrophic and nonphototrophic organisms belonging to group B, on the basis of the common history of the primary plastid endosymbiosis.

Key words: evolution, nuclear genes, phylogeny, plastids, primary endosymbiosis, secondary endosymbiosis

Problems in previous nuclear multigene phylogeny of the primary photosynthetic eukaryotes

The origin and diversity of plastids (chloroplasts) in eukaryotic cells can be attributed to two types of endosymbiotic events: primary endosymbiosis, and secondary or tertiary endosymbiosis. The green plants, glaucophytes and red algae are "primary photosynthetic eukaryotes", whose plastids may have originated directly from a cyanobacterium-like ancestor via "primary endosymbiosis" (*e.g.*, Bhattacharya & Medlin 1995, Delwiche 1999, McFadden 2001, Cavalier-Smith 2002a). By contrast, the plastids of other lineages of eukaryotic phototrophs appear to be the result of secondary

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or tertiary endosymbiotic events (involving a phototrophic eukaryote and a host cell) because they are surrounded by three or four bounding membranes, and the endosymbiotic remnant of the photosynthetic eukaryotic nucleus, called the nucleomorph, may be recognized between the bounding membranes (*e.g.*, Delwiche 1999, McFadden 2001, Cavalier-Smith 2002a, Yoon *et al.* 2002). Cavalier-Smith (1981, 2002b) classified only the primary photosynthetic eukaryotes as the kingdom "Plantae". All of the plastids in eukaryotes can be considered to be the product of a single primary endosymbiosis, based on the phylogenetic analyses of plastid-coding genes and the similarity of the plastid genome organization (*e.g.*, Morden *et al.* 1992, Nelissen *et al.* 1995, Bhattacharya & Medlin 1995) and the conserved mosaic origin of Calvin cycle enzymes in the red alga *Cyanidioschyzon* and green plants (Matsuzaki *et al.* 2004).

Recently, three phylogenetic studies of concatenated amino acid sequences of multiple nuclear genes were carried out (Moreira *et al.* 2000, Baldauf *et al.* 2000, Bapteste *et al.* 2002). However, these studies included only a single red algal OTU mainly derived from *Porphyra.* Although the red algae were traditionally assigned to the single class Rhodophyceae comprised of two subclasses, the Bangiophycidae (including *Porphyra* and *Cyanidioschyzon*) and the Florideophycidae (*e. g.* Bold & Wynne 1985), recent molecular phylogenetic analyses demonstrated that the Bangiophycidae are paraphyletic, and the red algae are composed of two sister clades (*e.g.*, Ciniglia *et al.* 2004), which can be assigned to the Rhodophyceae (including *Porphyra* and Florideophyceae) and Cyanidophyceae (including *Cyanidioschyzon* and other acid hot spring red algae). Baldauf *et al.* (2000) used four nuclear genes of a large number of OTUs from various eukaryotes. However, the phylogenetic position of the red alga was ambiguous, possibly because only a single OTU was analyzed and its sequences were incomplete. Moreira *et al.* (2000) demonstrated the strong monophyly of red and green plants based on 13 nuclear genes, but they used oomycete beta tubulin sequences and two cryptomonad nucleomorph (highly reduced red algal nucleus after the plastid secondary endosymbiosis) sequences for the red algal OTU. Therefore, nuclear gene sequences from free-living red algae, especially from the Cyanidiophyceae, were needed to resolve reliable phylogenetic positions of the three groups of the primary photosynthetic eukaryotes.

Macrophylogeny using nuclear gene sequences obtained from the genome project of the red alga *Cyanidioschyzon*

Complete sequences of all of the three genomes, mitochondrial (Ohta *et al.* 1998), plastid (Ohta *et al.* 2003) and nuclear (Matsuzaki *et al.* 2004) genomes, from *Cyanidioschyzon merolae* (Cyanidiophyceae) were determined for the first time in eukaryotic algae. Nozaki *et al.* (2003a) thus used complete sequences of the nuclear genes from *C. merolae* for deducing the natural phylogenetic relationships between the three primary photosynthetic eukaryotes. The alignment of four nuclear genes used by Baldauf *et al.* (2000) was used, but nucleomorph sequences and amitochondrial sequences were excluded because these highly divergent gene sequences seem to exhibit unusual substitutions, which can cause long-branch attraction or unnatural phylogenetic resolution (see Van de Peer *et al.* 1996, Stiller *et al.* 2001). Therefore, only mitochondria-containing eukaryotes were analyzed (Nozaki *et al.* 2003a).

Since it was generally considered that the amitochondrial eukaryotes represent the most basal eukaryotes (see Cavalier-Smith 1998) (Fig. 1), exclusion of such organisms from eukaryotic macrophylogenies seemed to result in difficulty for designating the outgroup. In order to resolve basal mitochondria-containing eukaryotes, however,

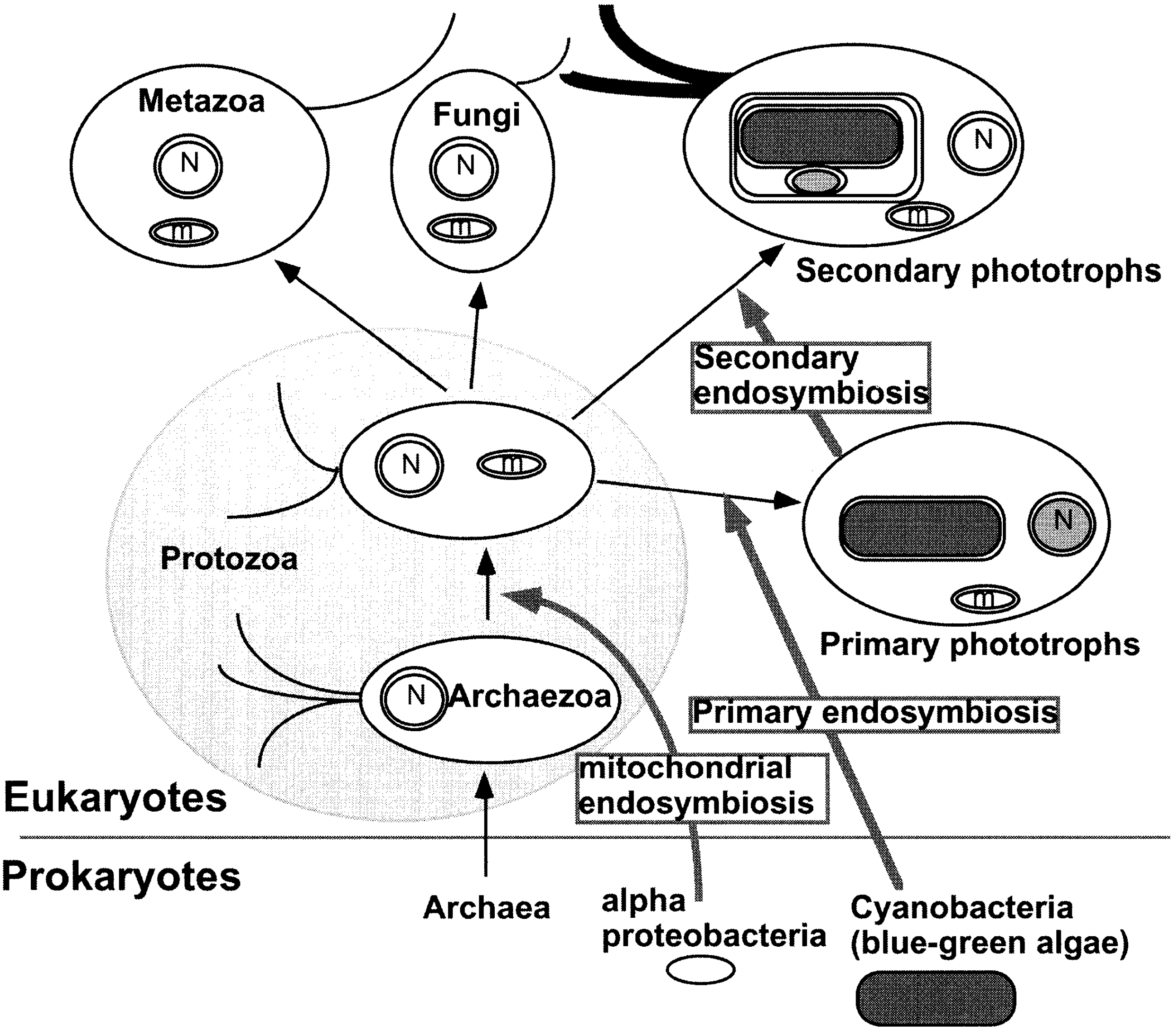

FIG. 1. Diagrams of plastid and mitochondrial endosymbioses and evolution of eukaryotes (based on Cavalier-Smith 1998). N, nucleus; m, mitochondrion.

Nozaki *et al.* (2003a) carried out the paralogous comparison of the combined data set from alpha- and beta-tubulin sequences. The alpha- and beta-tubulin genes may have originated from gene duplication, after the origin of eukaryotes (Edlind *et al.* 1996, Keeling & Doolittle 1996). Therefore, basal eukaryotic organisms can be deduced from a phylogenetic comparison of these two genes (see Iwabe *et al.* 1989). In order to increase the phylogenetic information, Nozaki *et al.* (2003a) aligned concatenated sequences of these two paralogous genes from 40 mitochondria-containing organisms with those of reversibly concatenated alpha- and beta-tubulin genes from the same 40 organisms. The alignment was carried out according to McKean *et al.* (2001). After removing the gaps, 560 amino acids from 80 OTUs, in total, were used for phylogenetic analyses (Nozaki *et al.* 2003a). Figure 2 shows the phylogenetic tree constructed using the concatenated amino acid sequences of the alpha- and beta-tubulin genes. Two identical subtrees are theoretically resolved. High bootstrap values resolved

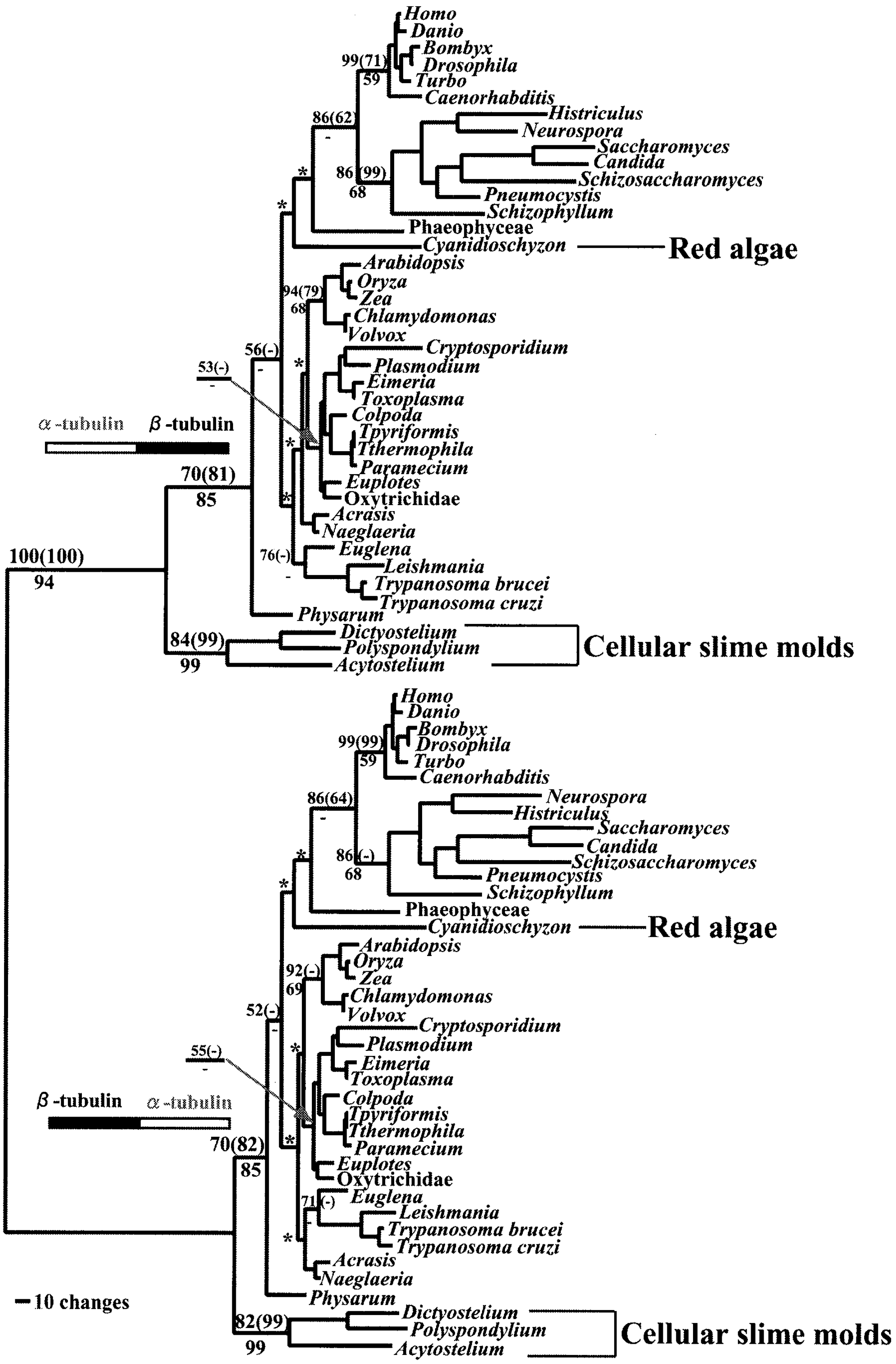

FIG. 2. One of the four most parsimonious trees of concatenated paralogous genes (alpha- and beta-tubulin genes) from 40 mitochondria-containing organisms based on the unambiguously aligned 560 amino acids, with reversibly concatenated alpha- and beta-tubulin genes from the same 40 organisms designated as the outgroup. Numbers without and with parentheses above branches are the bootstrap values (50% or more) based on 1000 replications of the full heuristic MP analysis and the neighbor-joining method, respectively. Branches resolved with 50% or more QPS values by quartet puzzling-maximum likelihood calculation. Asterisks indicate branches not supported by 50% or more bootstrap/ quartet puzzling support (QPS) values of the three phylogenetic methods. Bootstrap/QPS values supporting distal branches are not shown. (Based on Nozaki et al. (2003a); reproduced by permission of Springer-Verlag New York Inc.).

that the most basal position of the clade was held by three cellular slime molds. Therefore, the cellular slime molds were designated as the outgroup in our phylogenetic analyses of four nuclear genes (Nozaki *et al.* 2003a).

Recently, Cavalier-Smith (2002b) and Stechmann & Cavalier-Smith (2002) suggested that the Amoebozoa represent the most basal eukaryotic lineage, based on the diversification of the microtubular cytoskeleton and/or presence/absence of dihydrofolate reductase-thymidylate synthase (DHFR-TS) gene fusion. Therefore, the cellular slime molds appear to be one of the most basal eukaryotic organisms and are appropriate as the outgroup in phylogenetic analyses of mitochondria-containing organisms. Although Stechmann & Cavalier-Smith (2003) and Cavalier-Smith (2003) very recently suggested that the Opisthokonta (Metazoa and Fungi) and Amoebozoa represent a monophyletic group (unikonts), based on the unique fusion of three genes in the pyrimidine biosynthetic cluster (the CAD complex: carbamoyl-phosphate synthetase II, aspartate carbamoyltransferase, and dihydroorotase), this gene fusion is found in the nuclear genome of the red alga *Cyanidioschyzon merolae* (Matsuzaki *et al.* 2004, Nozaki *et al.* 2005).

To resolve the reliable phylogenetic position of the red algae within the eukaryotic organisms, Nozaki *et al.* (2003a) performed phylogenetic analyses of various eukaryotic, mitochondria-containing organisms based on a 1525-amino-acid sequence of four concatenated nuclear genes (actin, elongation factor-1alpha, alpha-tubulin and beta-tubulin). The analyses resolved the presence of two large monophyletic groups (groups A and B) and the basal lineage, Amoebozoa (true and cellular slime molds) (Nozaki *et al.* 2003a) (Fig. 3). Group A corresponded to the Opisthokonta (Metazoa and Fungi), whereas group B included various primary and secondary plastid-containing lineages (red algae, green plants, glaucophytes, euglenoids, heterokonts, and apicomplexans), Ciliophora, Kinetoplastida, and Heterolobosea, where the red algae represent the most basal group and the glaucophyte *Cyanophora* is the secondary basal lineage. The green plants are positioned distally within group B (Fig. 3). These phylogenetic positions are consistent with the fact that the glaucophytes and red algae have plastids with primitive features, such as phycobillisomes, common to the cyanobacteria (*e.g.*, Bold & Wynne 1985, South & Whittick 1987). Although the group B was not robustly resolved (Fig. 3), the monophyly of this group is supported by the presence of DHFR-TS gene fusion (Stechmann & Cavalier-Smith 2002, for red algae, see the sequence data of Matsuzaki *et al.* 2004).

A new scenario of plastid endosymbiosis and new concept of the "Plantae"

The phylogenetic results by Nozaki *et al.* (2003a) also suggested that the plastid primary endosymbiosis likely occurred once in the common ancestor of group B, and primary plastids were subsequently lost in the ancestors of the primary plastid lacking organisms belonging to this group (Fig. 4). Thus, Discicristata, Heterokonta and Alveolata might have experienced the primary endosymbiosis. Discicristata are characterized by having disk-shaped mitochondria cristae, and include Heterolobosea, Kinetoplastida, and the Euglenophyceae which contain the secondary plastids (Cavalier-Smith 2002b). Alveolata include Cilliophora and two secondary plastid-containing groups, namely, Apicomplexa and Dinoflagellates. (Cavalier-Smith 2002b). Although the three groups of the secondary phototrophs (Haptophyceae, Cryptophyceae and Chlorarchniophyceae) were not analyzed by Nozaki *et al.* (2003a), recent studies of the plastid-targeted glyceraldehyde-3-phosphate dehydrogenase genes demonstrated that the secondary plastids of the Haptophyceae, Cryptophyceae, Dinophyceae, Apicomplexa and Heterokonta have the single endosymbiotic origin (Harper &

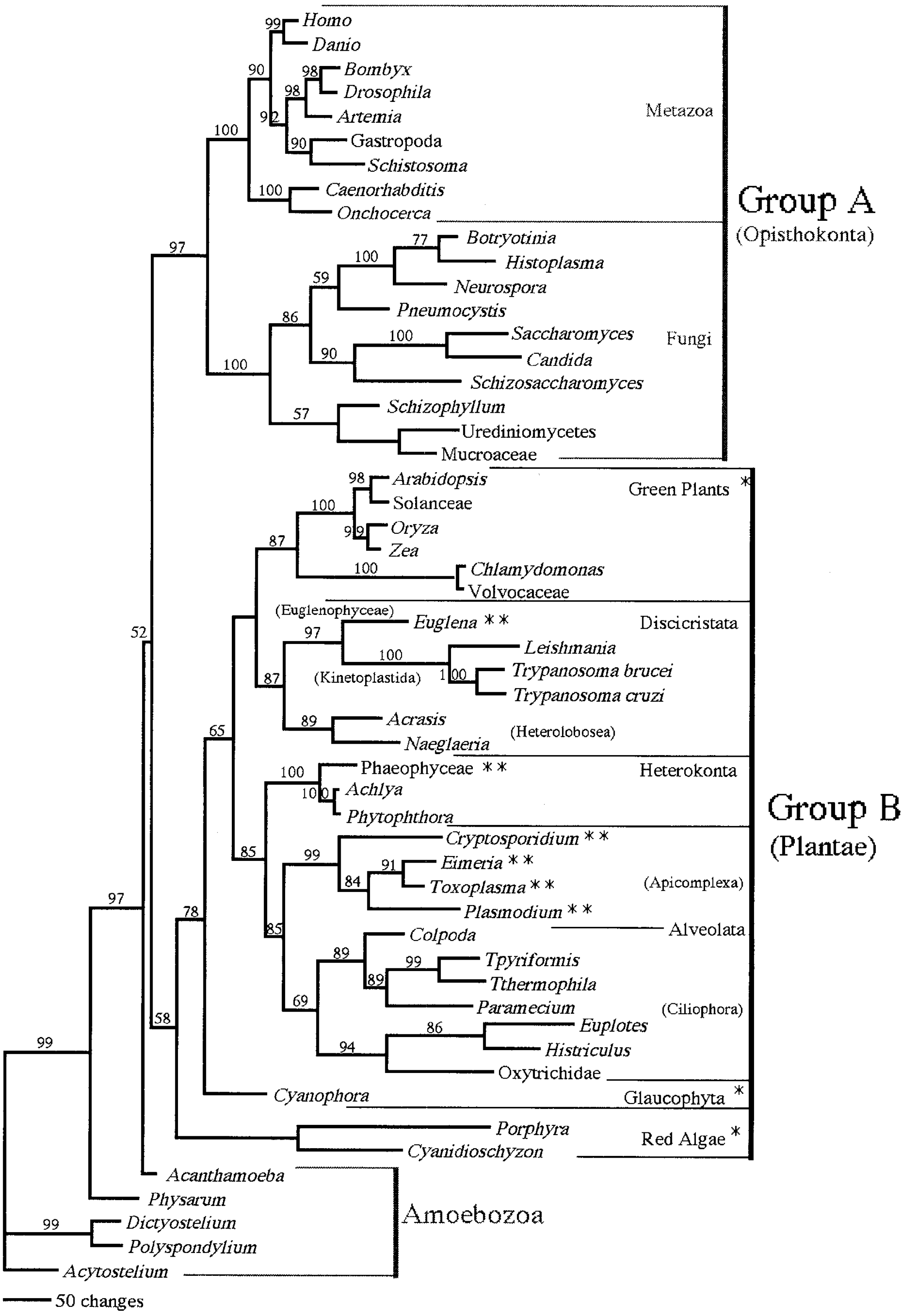

FIG. 3. One of the two most parsimonious tree (with tree length of 5221 and consistency index of 0.5417) based on 1525 amino acid sequences from the four nuclear genes (actin, elongation factor-1alpha, alpha-tubulin and beta-tubulin) of 53 OTUs representing a wide-range of mitochondria-containing eukaryotic taxa. Branch lengths are proportional to numbers of amino acid substitutions, which are indicated by the scale bar below the tree. Numbers above branches are the bootstrap values (50% or more) based on 1000 replications of the full heuristic analysis (with simple addition sequence). Single and double asterisks indicate the primary and secondary plastids, respectively. (Based on Nozaki et al. (2003a); reproduced by permission of Springer-Verlag New York Inc.).

Keeling 2003). Therefore, almost all of the secondary plastids appear to have evolved after the plastid primary endosymbiosis, based on the phylogenetic study by Nozaki *et al.* (2003a) (Figs. 3, 4). Therefore, establishment of the secondary endosymbiosis may be based on the experience of the plastid primary endosymbiosis. The genes that had contributed to the primary endosymbiosis might have also contributed to the secondary endosymbiosis.

Andersson & Roger (2002) demonstrated that the 6-phosphogluconate dehydrogenase (*gnd*) gene with cyanobacterial affinity are present in the nuclear genomes of photosynthetic eukaryotes as well as of plastid-lacking organisms of Heterokonta and Heterolobosea, and these genes form a monophyletic group that is sister to the cyanobacterial clade. Therefore, two alternative hypotheses were suggested. First, the *gnd* genes might have introduced to the nucleus from the cyanobacteria during the "ancient" primary endosymbiosis. Second, the secondary endosymbiosis might have mediated the gene transfer of *gnd*. The phylogenetic results by Nozaki *et al.* (2003a) are consistent with the first hypothesis and these cyanobacterial genes in the nuclear genome can be considered "relics" of the plastid primary endosymbiosis (Fig. 4).

Very recently, several cyanobacterial or plant-like genes were found in the plastid-lacking Kinetoplastida by Hannaert *et al.* (2003). Since the Euglenophyceae have secondary plastids and are closely related to the Kinetoplastida, Hannaert *et al.* (2003) discussed that a common ancestor of both Euglenophyceae and Kinetoplastida already acquired the secondary plastids that gave rise to the plant-like genes in the Kinetoplastida nuclear genome. Furthermore, Cavalier-Smith (2003) suggested the single, very ancient secondary endosymbiosis of green alga before divergence of Discicristata and Cercozoa (including the secondary photosynthetic eukaryotes Chlorarachniophyta) (Fig. 5). However, phylogenetic analyses of various euglenoid taxa suggested that the evolution of phototrophy occurred in the common ancestor of the monophyletic plastid-containing group (Euglenales) distally positioned within the Euglenophyceae (Montegut-Felkner & Triemer 1997, Preisfeld *et al.* 2000; Müllner *et al.* 2001), and the *Heterolobosea* are positioned outside the clade composed of Euglenophyceae and Kinetoplastida (Baldauf *et al.* 2000, Nozaki *et al.* 2003a) (Figs. 3, 4). In addition, the cladistic analysis of plastid gene losses suggested the relatively recent acquisition of the secondary plastid of *Euglena* (after the divergence of Chlorophyceae and Trebouxiophy-ceae) (Nozaki *et al.* 2003b). Therefore, the plastid secondary endosymbiosis can hardly be considered before the divergence of Euglenophyceae and Kinetoplastida or Heterolobosea, and the cyanobacterial or plant-like genes found in Heterolobosea (Andersson & Roger 2002) and Kinetoplastida (Hannaert *et al.* 2003) are possibly derived from the ancient primary plastid endosymbiosis suggested by Nozaki *et al.* (2003a) (Fig. 4). Thus, Nozaki *et al.* (2003a) proposed a new concept of "Plantae" for the large monophyletic group resolved (group B, Fig, 3), based on the common history of the plastid primary endosymbiotic event (Fig. 4). The Planatae include primary plastid-containing phototrophs and eukaryotes that possibly contain cyanobaterial genes acquired in the primary endosymbiosis. Since the "Plantae" sensu Cavalier-Smith (1981, 2002b) (only the primary plastid-containing eukaryotes) are apparently paraphyletic, the concept of "Plantae" of Nozaki *et al.* (2003a) is more natural and based on the important evolutionary event (plastid primary endosymbiosis) during the eukaryotic evolution. The members of the Plantae emended by Nozaki *et al.* (2003a) are very similar to those of the Bikonta (bikonts) recently proposed by Stechmann & Cavalier-Smith (2002, 2003) and Cavalier-Smith (2003) (Fig. 5), who classified the eukaryotes into the Amoebozoa, Opisthokonta, and Bikonta, on the basis of cell morphology and synapomorphic changes in gene organization (insertion, gene fusion,

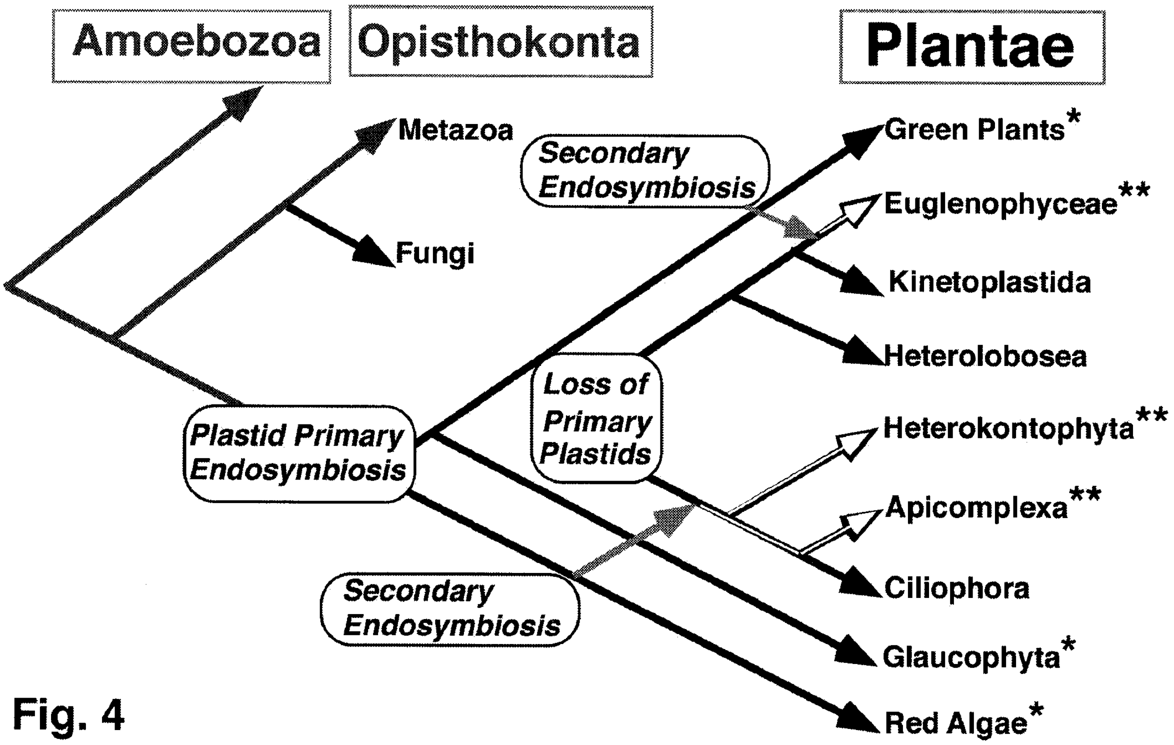

Fig. 4

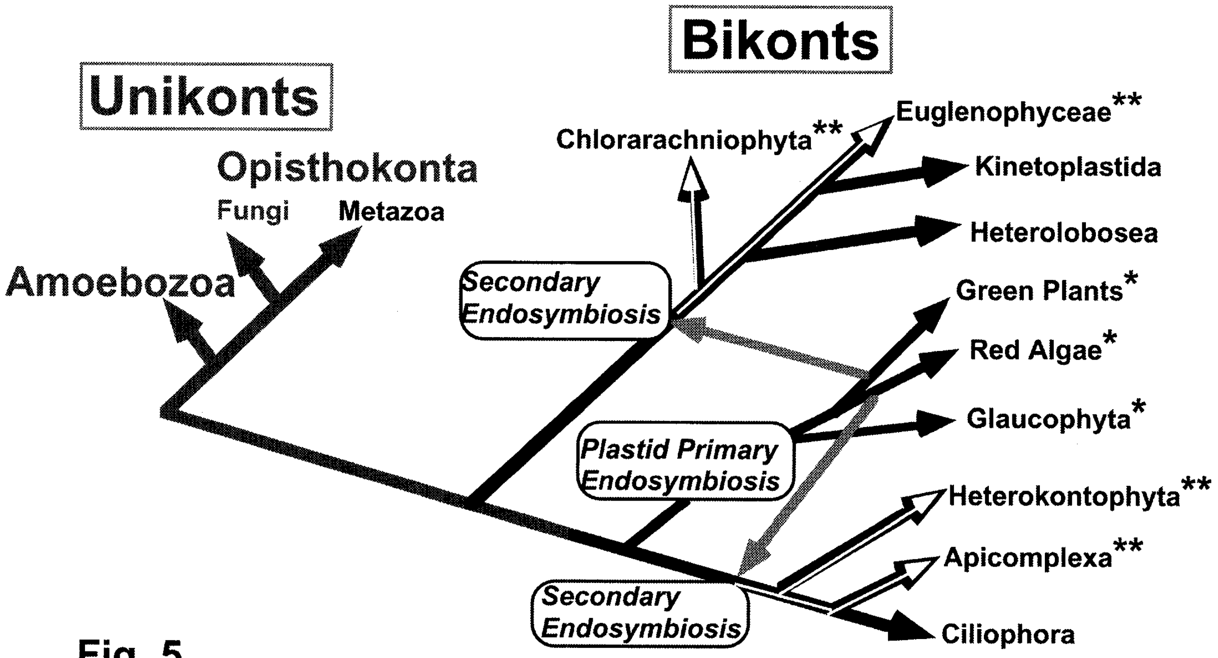

Fig. 5

FIGS. 4, 5. Two recent hypotheses of the origin and evolution of plastids. Single and double asterisks indicate the primary and secondary plastids, respectively. 4: Based on Nozaki *et al.* (2003a). 5: Based on Cavalier-Smith (2003).

etc.) (Fig. 5). However, evolutionary events of the primary plastids after the primary endosymbiosis are essentially different between the Plantae (Fig. 4) and Bikonta (Fig. 5). In the Bikonta, the plastid primary endosymbiosis have been experienced in the ancestor of only the three groups of the primary photosynthetic eukaryotes because the three groups are still believed to be monophyletic (Fig. 5).

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References

Andersson, J. O. & A. J. Roger. 2002. A cyanobacterial gene in nonphotosynthetic protests - An early chloroplast acquisition in Eukaryotes? Curr. Biol. 12: 115-119.

Baldauf, S. L., A. J. Roger, I. Wenk-Siefert & W. F. Doolittle. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290: 972-977.

Bapteste, E., H. Brinkmann, J. A. Lee, D. V. Moore, C. W. Sensen, P. Gordon, L. Duruflé, T. Gaasterland, P. Lopez, M. Müller & H. Philippe. 2002. The analysis of 100 genes supports the grouping of three highly divergent amoebae: *Dictyostelium, Entamoeba*, and *Mastigamoeba*. Proc. Natl. Acad. Sci. USA 99: 1414-1419.

Bhattacharya, D. & L. Medlin. 1995. The phylogeny of plastids: A review based on comparisons of small subunit ribosomal RNA coding regions. J. Phycol. 31: 489-498.

Bold, H. C. & J. M. Wynne. 1985. Introduction to the Algae. 2nd edn. Prentice-Hall. Inc., Englewood Cliffs, New Jersey.

Cavalier-Smith, T 1981. Eukaryote kingdoms, seven or nine? BioSystems 14: 461-481.

——. 1998. Nemomonada and the origin of animals and fungi. In: Coombs, G. H. & K. Vickerman. (eds.) Evolutionary Relationships among Protozoa., pp. 375-407, Kluwer Academic Publishers, Dordrecht.

——. 2002a. Chloroplast evolution: Secondary symbiogenesis and multiple losses. Curr. Biol. 12: R62-R64.

——. 2002b. The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. Int. J. Syst. Evol. Microbiol. 52: 297-354.

——. 2003. The excavate protozoan phyla Metamonada Grasse emend. (Anaeromonadea, Parabasalia, Carpediemonas, Eopharyngia) and Loukozoa emend. (Jakobea, Malawimonas): their evolutionary affinities and new higher taxa. Int. J. Syst. Evol. Microbiol. 53:1741-1758.

Ciniglia, C., H. S. Yoon, A. Pollio, G. Pinto & D. Bhattacharya. 2004. Hidden biodiversity of the extremophilic Cyanidiales red algae. Mol. Ecol. 13: 1827-1838.

Delwiche, C. F. 1999. Tracing the thread of plastid diversity through thetapestry of life. Am. Nat. 154: 164-177.

Edlind, T. D., J. Li, G. S. Visvesvara, M. H. Vodkin, G. L. McLaughlin & S. K. Katiyar. 1996. Phylogenetic analysis of beta-tubulin sequences from amitochondrial protozoa. Mol. Phylogenet. Evol. 5: 359-367.

Hannaert, V., E. Saavedra, F. Duffieux, J. P. Szikora, D. J. Rigden, P. A. M. Michels & F. R. Opperdoes. 2003. Plant-like traits associated with metabolism of *Trypanosoma* parasites. Proc. Natl. Acad. Sci. USA 100: 1067-1071.

Harper, J. T. & P. J. Keeling. 2003. Nucleus-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) indicates a single origin for Chromalveolate plastids. Mol. Biol Evol 20: 1730-1735.

Iwabe, N., K. Kuma, M. Hasegawa, S. Osawa & T. Miyata. 1989. Evolutionary relationship of archaebacteria, eubacteria, and eukaryotes inferred from phylogenetic trees of duplicated genes. Proc. Natl. Acad. Sci. USA 86: 9355-9359.

Keeling, P. J. & W. F. Doolittle. 1996. Alpha-tubulin from early-diverging eukaryotic lineages and the evolution of the tubulin family. Mol. Biol. Evol. 13: 1297-1305.

Matsuzaki, M., O. Misumi, T. Shin-i, S. Maruyama, M. Takahara, S. Y. Miyagishima, T. Mori, K. Nishida, F. Yagisawa, K. Nishida, Y. Yoshida, Y. Nishimura, S. Nakao, T. Kobayashi, Y. Momoyama, T. Higashiyama, A. Minoda, M. Sano, H. Nomoto, K. Oishi, H. Hayashi, F. Ohta, S. Nishizaka, S. Haga, S. Miura, T. Morishita, Y. Kabeya, K. Terasawa, Y. Suzuki, Y. Ishii, S. Asakawa, H. Takano, N. Ohta, H. Kuroiwa, K. Tanaka, N. Shimizu, S. Sugano, N. Sato, H.

Nozaki, N. Ogasawara, Y. Kohara & T. Kuroiwa. 2004. Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. Nature 428: 653-657.

McFadden, G. I. 2001. Primary and secondary endosymbiosis and the origin of plastids. J. Phycol. 37: 951-959.

McKean, P. G., S. Vaughan & K. Gull. 2001. The extended tubulin superfamily. J Cell Sci 114: 2723-2733.

Montegut-Felkner, A. E. & R. E. Triemer. 1997. Phylogenetic relationships of selected euglenoid genera based on morphological and molecular data. J. Phycol. 33: 512-519

Morden, C. W., C. F. Delwiche, M. Kuhsel & J. D. Palmer. 1992. Gene phylogenies and the endosymbiotic origin of plastids. BioSystems 28: 75-90.

Moreira, D., H. Le Guyader & H. Philippe. 2000. The origin of red algae and the evolution of chloroplasts. Nature 405: 69-72.

Müllner, A. N., D. G. Angeler, R. Samuel, E. W. Linton & R. E. Triemer. 2001. Phylogenetic analysis of phagotrophic, phototrophic and osmotrophic euglenoids by using the nuclear 18S rDNA sequence. Int. J. Syst. Evol. Micr. 51: 783-791

Nelissen, B., Y. Van de Peer, A. Wilmotte & R. De Wachter. 1995. An early origin of plastids within the cyanobacterial divergence is suggested by evolutionary trees based on complete 16S *r*RNA sequences. Mol Biol Evol 12: 1166-1173.

Nozaki, H., M. Matsuzaki, O. Misumi, H. Kuroiwa, T. Higashiyama & T. Kuroiwa. (in press) Phylogenetic implications of the CAD complex from the primitive red alga *Cyanidioschyzon merolae* (Cyanidiales, Rhodophyta). J. Phycol. 41.

——, M. Matsuzaki, M. Takahara, O. Misumi, H. Kuroiwa, M. Hasegawa, T. Shin-i, Y. Kohara, N. Ogasawara & T. Kuroiwa. 2003a. The phylogenetic position of red algae revealed by multiple nuclear genes from mitochondria-containing eukaryotes and an alternative hypothesis on the origin of plastids. J. Mol. Evol. 56: 485-497.

——, N. Ohta, M. Matsuzaki, O. Misumi & T. Kuroiwa. 2003b. Phylogeny of plastids based on cladistic analysis of gene loss inferred from complete plastid genome sequences. J. Mol. Evol. 57: 377-382.

Ohta, N., M. Matsuzaki, O. Misumi, S. Miyagishima, H. Nozaki, K. Tanaka, T. Shin-i, Y. Kohara & T. Kuroiwa. 2003. Complete sequence and analysis of the plastid genome of the unicellular red alga *Cyanidioschyzon merolae*. DNA Res. 10: 67-77.

——, N. Sato & T. Kuroiwa. 1998. Structure and organization of the mitochondrial genome of the unicellular red alga *Cyanidioschyzon merolae* deduced from the complete nucleotide sequence. Nucleic Acids Res. 26: 5190-5198.

Preisfeld, A., S. Berger, I. Busse, S. Liller & H. G. Ruppel. 2000. Phylogenetic analyses of various euglenoid taxa (Euglenozoa) based on 18S *r*DNA sequence data. J. Phycol. 36: 220-226.

South, G. R. & A. Whittick. 1987. Introduction to Phycology. Blackwell Sci. Pub., Oxford.

Stechmann, A. & T. Cavalier-Smith. 2002. Rooting the eukaryote tree by using a derived gene fusion. Science 297: 89-91.

—— & ——. 2003. The root of the eukaryote tree pinpointed. Curr .Biol. 13: R665-R666.

Stiller, J. W., J. Riley & B. D. Hall. 2001. Are red algae plants? A critical evaluation of three key molecular data sets. J. Mol. Evol. 52: 527-539.

Van de Peer, Y., S. A. Rensing, U. G. Maier & R. De Wachter. 1996. Substitution rate calibration of small subunit ribosomal RNA identifies chlorarachniophyte endosymbionts as remnants of green algae. Proc. Natl. Acad. Sci. USA 93: 7732-7736.

Yoon, H. S., J. D. Hackett & D. Bhattacharya. 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. Proc. Natl. Acad. Sci. USA 99: 11724-11729.

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